TRACE ELEMENTS PROFILE IN VIPER ENVENOMATION

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CERTIFICATE

This is to certify that this dissertation titled **"TRACE ELEMENTS PROFILE IN VIPER ENVENOMATION"** submitted by Dr. P. RAVIKUMAR to the faculty of General Medicine, The Tamilnadu Dr. M.G.R. Medical University, Chennai in partial fulfillment of the requirement for the award of MD degree Branch I (General Medicine) is a bonafide research work carried out by him under our direct supervision and guidance.

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DECLARATION

I, Dr. P.RAVIKUMAR, solemnly declare that the dissertation titled **"TRACE ELEMENTS PROFILE IN VIPER ENVENOMATION"** has been prepared by me.

This is submitted to the Tamil Nadu Dr. M.G.R. Medical University, Chennai, in partial fulfillment of the regulations for the award of MD Degree Branch I (General Medicine).

It was not submitted to the award of any degree/ diploma to any University either in part or in full form previously.

Place : Madurai

Date :

Dr. P.RAVIKUMAR

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ABBREVIATIONS AND ACRONYMS

AMPase – Adenosine monophosphatase
APC – Antigen presenting cell
APP – Acute phase protein
APR – Acute phase reaction
ASV – Anti snake venom
ATPase – Adenosine triphosphatase
cAMP – Cyclic AMP
CPK – Creatine phosphokinase
CRP – C-Reactive protein
DHL– apo A1- High density lipoprotein-apolipoprotein A1
DIC – Disseminated Intravascular Coagulation
DNase – Deoxy ribonuclease
ECM – Extracellular matrix
HR – Hemorrhagins
ICAM – Intercellular adhesion molecule
IL – Interleukin
IL-1–RacP – IL-1 receptor accessory protein
LFA – Leucocyte function antigen
LT – Leucotriene
MBP – Mannose binding protein
MCAF – Monocyte chemoattractant factor
NADase – Nicotinamide adenine dinucleotidase
NGF – Nerve growth factor
PAF – Platelet activating factor
PGE2 - Prostaglandlin E2
PLA2 – Phospholipase A2
RA – Rhematoid arthritis
TfR – Transferrin receptor
TGF β – Transforming growth factor β
TNF – Tumor necrosis factor
TTR – Transthyretin
VCAM- Vascular cell adhesion molecule
VLA – Very late antigen

CONTENTS

INTRODUCTION

Snake envenomation is one of the important environmental and occupational problems in India and other developing countries where there are lots of lands for agricultural work. The incidence is 2, 00,000 per year in India of which 15,000 die due to the complications of envenomation (Swaroop and Garb, 1954). It is also a social problem, where cases are mismanaged in the hands of the native traditional medical practitioners with lots of false beliefs regarding snake bite. This also accounts for the time lag for the patients to reach the hospital for definitive management.

The expenses that are put forth for the treatment of snake envenomation and its complications are enormous. It consumes a major chunk of budget allotted for health by the government. Apart from a general statistics of snake bite and envenomation, this study also analyses the changes in trace elements especially zinc, magnesium and iron during envenomation, an acute inflammatory state.

Snake envenomation alters the internal biochemical milieu of the body which is due to inflammatory response induced by the combined effect of the toxins present in the venom predominantly, and to a lesser extant due to infection produced by bacterial flora in the mouth of the snake. This proceeds as a vicious cycle. These alterations may be influenced by the genetic susceptibility, chemical constituents, quantity and metabolic degradation of the venom, and the surface area of the patient. There is a possibility for these alterations to produce changes in the trace element levels in the serum. Hence the hypothesis considered for the present study is “Trace elements level is not affected by inflammatory response”. However, the proposed study will also help to confirm or refute the published reports on the effects of inflammation on trace elements.

REVIEW OF LITERATURE

Snake envenomation

Ophitoxemia is the term that characterizes the clinical spectrum of snake envenomation. Of the 2500-3000 species of snakes distributed worldwide, about 500 are venomous. Based on their morphological characteristics including arrangement of scales, dentition, osteology, myology, sensory organs, etc., snakes are categorized into families. The families of venomous snakes are Atractaspididae, Elapidae, Viperidae and Hydrophidae (Warrell, 1996). The major families in India are:

Elapidae - cobra and krait.

Viperidae-Russell's viper, pit viper, saw scaled viper.

Hydrophidae-sea snakes(less common).

Of the 52 poisonous species in India (Kochva, 1978), majority of the bites are attributed to 5 species:

King cobra - Ophiophagus hannah.

Common cobra - Najanaja.

Russell's viper - Daboia russellii.

Saw scaled viper - Echis carinatae.

Common krait - Bungarus caeruleus.

Identification of snakes:

Kochva (1978) studied the anatomy of snakes including their oral glands and fangs (venom apparatus) which are mainly used for the identification of them.

1) Elapidae:

The fangs are small and immobile. The ventral scales cover the body completely.

2) Viperidae:

They have a triangular head with tiny head shields. The front fangs are big, hinged (reverse fangs), erectile, canaliculated and covered by sheath. They are curved and have a wide range of motion.

The pupils are vertical. The dorsum has different colors. The ventral surface is white with black spots at the two ends of scales.

In pit viper, a pit is seen beyond the nasal shield.

3) Sea snakes:

They have a sky blue color dorsally and white color ventrally. The body is compressed posteriorly. The tail being flat is shaped like a paddle and the body is round.

Venom:

The approximate amount of venom inoculated per bite and the lethal dose is as follows:

Type of snake	quantity per bite	lethal dose
Cobra	200mg	120mg
Krait	20mg	60mg
R.viper	150mg	150mg
S.viper	5mg	80mg

The maximum output of venom is in summer and monsoon which leads to increased mortality. In winter, venom is viscous and small in quantity. They secrete about 10% of the available venom per bite, except R.viper, which exhausts about ¾ of the venom in the gland with the first strike. There is no support for the belief that snakes are less dangerous after they have taken a prey.

1 ml of Haffkins institute ASV neutralizes about 0.6 mg of venom of all the four snakes.

Chemical characters and composition:

The venom is acidic with a specific gravity of 1030-1070. It is crystalline in nature (needle shaped crystals), and is soluble in water. It consists of enzymatic and non enzymatic proteins. It is not toxic when taken orally as it is degraded by enzymes of GIT. Most venom get bound to kidney and excreted through them. A few get excreted through bile. Mostly they do not cross the blood brain barrier (Parrish, 1980).

Enzyme proteins:

They have a facilitator effect in the spread of venom.

1) **Hydrolytic**- hydrolase, lecithinase, hyaluronidase, exopeptidases, DNase, phosphatases, exonucleases, pyrophosphatase(ATPase, NADase) and 5'nucleotidase(AMP ase)
2) **Non hydrolytic**-L-amino acid oxidase.

Enzymes specific to viper:

These include arginine ester hydrolase, kininogenase, factor X and prothrombin activating enzyme.

Enzymes specific to Elapid:

They are acetyl choline esterase, alkaline/acid phosphomonoesterase, phospho lipase-B and glycerophosphatase.

Non enzyme proteins:

They have the lethal effect.

Those of Viper include nerve growth factor (NGF) and hemorrhagins, and of Elapids are nerve growth factor, polypeptide neuro and cardio toxins.

a) Hemorrhagins - There are two types of hemorrhagins as HR1 and HR2. They are acidic, non proteolytic and, heat and pH stable.

b) NGF - It stimulates and control growth of sensory and sympathetic nerve cells.

c) Neurotoxins - They include: i) cobra toxin (cobra),

ii) α,β,γ bungarotoxins (krait) and

iii) erabutoxin (sea snakes).

There are no free –SH groups and they are always bridged which is important for their toxic properties.

EPIDEMIOLOGY:

Incidence:

Snake bite is a common medical emergency and is an occupational hazard in India. Around 15,000 people die out of nearly 2, 00,000 bites every year. Myint-Lwinet et al., (1985), who worked in Burma, found that 3.3 deaths/lak populations has occurred in the Burmian farmer groups which is as high as 5^{th} most common cause of all deaths. But the exact incidence cannot be estimated in developing countries due to improper reporting system. The precise estimate of incidence is possible in industrialized countries as found by Parrish et al (1980).

Patient factors:

Age:

The spectrum of age group commonly involved is 11-50 years with fewer incidences in young children and elderly. This may be due to reduced outdoor activity in this age group where there is increased possibility of snake bite (Sawai, Homa, 1974).

Sex:

Males sustain more bites in the ratio of 3:1, as their outdoor activity is more when compared to females (Sawai, Homa, 1974).

Part of the body involved:

Lower extremity is involved much, about 70%, of which foot is involved in 40%. Next comes to upper extremities about 25% with fingers involved in 13%.

Environmental factors:

Season:

The amount of venom is less during winter months as it is highly viscous. So the morbidity and mortality is less during winter. Also the incidence is less during winter as there is less of agricultural work. The incidence is more during warmer months and also during monsoon due to increased egress of snakes from their dwelling. It is also increased during ploughing and harvesting times.

Time of bite:

Usually snake bite occurs during hours of working from morning 6 am to late evening.

Place of bite:

It has been observed that snakes do not ingress human dwelling except for kraits which will enter homes and bite. Rural population is the most affected when compared to semi urban and urban dwellings. The main place of snake bite is the agricultural fields, followed by bushy areas and forests when people are indulged in clearing the bushes.

Clinical features

About 25% to 30% of poisonous snake bites do not result in envenomation because of

1. failure of venom gland –fang mechanism,
2. insufficiency of venom and
3. indirect injury with the fangs which results in minor scratches.

Local features:

The earliest symptom is pain, followed by bleeding from fang marks. Swelling follows that, extending proximally going for regional lymphangitis and adenitis.

Pain:

It is more severe with viper bite. Cobra bite causes burning sensation.

Swelling and bruising:

It occurs due to increased permeability of vessels caused by phospholipids, proteases, membrane damaging polypeptides, toxins, endogenous autocoids eg. histamine, 5-hydroxy tryptamine, kinins. It is less with cobra and krait. Skip lesions are common with cobra. Viper bites causes more cellulitis with ecchymosis with blood stained discharge through the fang marks. Absence of swelling even after 2 hours shows no envenomation.

Tissue necrosis:

It is due to myotoxic and lipolytic factors, ischaemia from thrombosis, compartment syndrome or tourniquet application (Moura- da- silva et al, 1996).

Lymphadenitis:

It is a sign of impending systemic envenomation.

General Systemic features:

Vomiting, diarrhea, colic, angioedema, wheeze, syncope can occur. Psychological symptoms due to fright may resemble neurotoxicity where patients can have flushing, dizziness, breathlessness, constricting chest pain, palpitations, sweating, acroparaesthesia. A clue to these symptoms to be psychological is that it appears too soon and proceeds more rapidly than the minimum time taken for neurotoxicity to occur.

Neurotoxicity:

Symptoms:

They include vomiting, heaviness of lids, blurred vision, paraesthesia around mouth, hyperacusis, headache, dizziness, vertigo, hypersalivation, congested conjunctiva and goose flesh.

Signs:

Ptosis is the earliest sign followed by external ophthalmoplegia. There is also palatal and pharyngeal paralysis, dysphonia, and flaccid limb paralysis. Respiratory paralysis results in drowsiness, convulsions, and coma. These effects wear off spontaneously by 1-7 days.

Tensilon test performed differentiates presynaptic and postsynaptic toxicity. It is done by administering 0.6 mg of atropine on glycopyrollate i.v, followed by 10mg edrophonium i.v. post synaptic toxicity will improve but not the presynaptic.

With sea snakes there will be myotoxicity which presents as thick swelling of tongue, stiffness, aches and tenderness of muscles, trismus which will proceed to generalized flaccid paralysis. Myoglobinuria, hyperkalaemia occurs in 3-8 hours as a result of rhabdomyolysis which can result in acute renal failure (Philips, 1988).

Cardiotoxicity:

It presents with hypotension, arrythmias and cardiac arrest.

Haemotoxicity :

Hemorrhagic bleb with uncontrolled bleed from the bite site is the earliest sign and is diagnostic of hemotoxicity of viper. Purpura, hematoma or generalized ecchymosis can occur. Painful large ecchymosis, purpura gangrenosa of lips, tips of nose, finger and toes can occur. Conjunctival and facial edema can occur.

Mucosal bleed in the form of hematuria, hemoptysis, gingival bleed, hematemesis and malena can occur. One should look for bleed in each gingical sulcus.

Cerebral hemorrhage, Sheehan's syndrome (Warrel, 1996) are also part of the reported hemotoxic manifestations.

Hypotension due to hypovolemia secondary to internal bleed, vasodilation, local inflammation and myocardial dysfunction can occur. Hemolysis is not common with Indian snakes (hemoglobinuria and jaundice).

Hypoalbuminemia, albuminuria, serous effusions and pulmonary edema can occur. Pulmonary and coronary thromboembolism can cause early collapse. Renal ischemia can cause loin pain and oliguria (Ratcliffe, 1989).

The time taken for features of envenomation to become evident and the mean time of death following envenomation is as follows:

	Onset	Death (without treatment)
Neurotoxic	6½ hrs (20min - 15hrs)	Cobra – 8hrs; krait - 18hrs
Hemotoxic	15½ hrs (2 - 24hrs)	R.viper - 3days S.viper - 5days
Cardiotoxic	½ hr (1/4 - 2hrs)	12 hrs

In hospital management:

All patients require admission and observation for at least 24 hours. All the vital parameters are checked initially and then as frequently as required based on which progression of disease is assessed and the appropriate treatment is planned (Malasit, 1986).

Available data and observations clearly show variations in symptoms, signs, clinical course, complications and recovery. Reasons for all such variations may be attributed to molecular basis of systemic inflammatory response syndrome related to the release of cytokines following severe envenomation. In the ensuing paragraphs these details are provided.

CYTOKINES:

The communication between numerous cell types involved in inflammation occurs through a complex network of molecular mediators-the cytokines that direct all stages of response from initiation to repair. They act in autocrine, paracrin and endocrine manner. They include polypeptides, neuropeptides, lipids, vasoactive amines, nucleotides, metabolites of oxygen and nitrogen.

GENERAL CHARACTERISTICS OF CYTOKINES:

Cytokines are a diverse group of proteins of relatively low-molecular weight (rarely more than 8-25 k Da) with multiple functions (Blackwill, 1989). They regulate important biological processes, such as cell growth and differentiation, cell activation, inflammation, immunity, tissue repair, fibrosis, and morphogenesis. Although cytokines are considered to be a "family", this is a functional rather than a structural concept; these proteins are not all chemically related. Cytokines are produced by immune cells and other cells when challenged with various environmental or inflammatory insults, and these molecules are the soluble message of cell communications (Hoch et al, 1993). The functions of cytokines are often redundant, and they can influence the synthesis and action of other cytokines, creating the "cytokine-network". Cytokines are effective at low concentrations (at a few pg/ml). This is due to their mode of action, involving binding to high-affinity receptors on the cell surface, which transmit cytokine signals to the nucleus. Cytokines often consist of a single chain and most cytokine receptors are made up of a single chain that binds to the cytokine, but does not contain signal transduction motifs. It has recently been reported that after binding of cytokines to their receptors, signal

transducing chains are bound to this complex, initiating cell activation. In many cases, signal transduction chains are shared by numerous cytokines, and families of cytokine receptors have been characterized based on similarities in their extracellular domains. Various T helper cell cytokines like IL-1, IL-6 and TNF-α are involved in the mediation of hypersensitivity reactions to venom components (Akira et al, 1990).

CHEMOKINES:

Chemokines are small polypeptides (8-10 kDa) that are synthesized by several cells, such as phagocytes, endothelial cells, keratinocytes in the skin, fibroblasts, and smooth muscle cells of connective tissues, as well as T helper cells and platelets (Miller, 1992).

OTHER INFLAMMATORY MEDIATORS:

In addition to cytokines, there are other important mediators that participate in inflammatory responses and allergic reactions that are relevant to venomous bites. These mediators are released by macrophages, and other tissue-resident cells, such as mast cells, neutrophils, eosinophils, basophils, lymphocytes and platelets (Dayer, 1994). This group includes a variety of molecules such as arachidonic acid metabolites-prostaglandins, thromboxanes, leukotrienes, particularly leukotriene B4 (LTB4), platelet-activating factor (PAF), oxygen free radicals, nitric oxide and vasoactive amines. Plasma contains four mediator-producing systems such as the kinine system, clotting system, fibrinolytic system and complement system. Following the activation of these systems, endothelial damage can induce activation of plasma clotting factors, resulting in increased vascular

permeability, vasodilatation, neutrophil chemotaxis and smooth-muscle contraction (Marshall and Herrmann, 1983). The activation of the complement system, both by classical and alternative pathways, contributes to the inflammatory mediators C5a (the most potent), C3a, and to a lesser extent, C4a (Vogt,1990). C5a, apart from being an inflammatory mediator by its own, also activates mast cells, inducing the release of their granule contents including histamine, serotonin, and LTB4. However, it should be emphasized that these mediators cooperate with cytokines in the control of inflammation.

SEQUENTIAL INVOLVEMENT OF CYTOKINES IN ACUTE INFLAMMATORY RESPONSES:

Alarm and secondary cytokines:

The cell most commonly associated with the initiation of tissue inflammatory responses is the macrophage (Larsen, 1983). Activated macrophages release a broad spectrum of cytokine and inflammatory mediators; the IL-1 and TNFα being of utmost importance for the initiation and propagation of the inflammatory process. At the inflammation site other cellular events, such as mast cell degranulation and platelet aggregation and activation can also result in the release of mediators, which are chemotactic for macrophages and monocytes and activate their functions.

IL-1 and TNFα (Gearing et al, 1994) are especially important in initiating the next series of reactions. As IL-1β is active in its secreted form, its effects on inflammatory responses are more widespread and prominent than those of IL-1α which is active in the cytosol or as a cell-associated form (Aderka et al, 1994). IL-1 and TNFα are

considered as early or "alarm" cytokines with pleiotropic activities, acting both locally and distally. However, the most important activity of these cytokines is the induction in stromal cells (fibroblasts) of a second wave of cytokines. This amplifies the inflammatory signal and mediates the various phenomena that are seen in the inflammatory process. The cytokines of this second wave include IL-6, chemokines, IL-1 and TNF (Barraviera et al, 1971). IL-1 and TNF are also capable of inducing their own production. The synthesis of a wide array of non-cytokine inflammatory mediators is also induced by IL-1 and TNF in macrophages and other tissue-resident or stromal cells. IL-1, TNF and IL-6 are considered the most important pro-inflammatory cytokines, as they produce a wide spectrum of biological activities that help coordinate the body responses to infection (Akira, 1992).

Pro-inflammatory cytokines induce local and systemic inflammatory manifestations. The local effects include the activation of vascular endothelium, increase in vascular permeability, and access of leukocytes into the affected tissue and their activation and local tissue destruction. The systemic manifestations include fever, the acute-phase response and induction of a systemic shock in severe inflammatory processes (Miller, 1992).

Vascular effects and cell exudation:

The first cytokine-mediated inflammatory manifestations include the dilatation and leakage of blood vessels, particularly the post-capillary venules. This results in tissue edema and, in some cases, red-cell extravasation, manifested by tissue redness. IL-1 and TNF stimulate blood flow, increasing vascular permeability and endothelial adhesiveness

for white blood cells and platelets (Butcher, 1991). Local release of cytokines leads to an influx of fluid, cells, and proteins that participate in host defenses at the inflammation site. Later the small vessels clot, preventing the spread of infection or inflammation in the blood and the residual fluid drains to regional lymph nodes, where an adaptive immune response is initiated. In addition, IL-1 and TNF-induced low molecular-weight mediators released by the inflamed tissue, including reactive oxygen species, nitrous oxide, and metabolites of arachidonic acid contribute to the vasoconstriction and vasodilatation of the blood vessels. Local effects of inflammation caused by the release of histamine, serotonin and platelet-activating factor also contribute to these vascular effects.

Leukocyte exudation and accumulation at the inflammation site involve changes in the adhesion patterns of leukocytes to endothelial cells, ultimately resulting in a tight binding of leukocytes to endothelial cells and their migration into the inflamed tissue. IL-1 and TNF induce the expression of adhesion molecules, such as the intercellular adhesion molecule-1 (ICAM-1) and the vascular intercellular adhesion molecule-1 (VCAM-1) on endothelial cells which bind with high affinity to their counter receptors on leukocytes, leukocyte function antigen-1 (LFA-1) and the very late antigen-4 (VLA-4), respectively. The interaction of these adhesion molecules arrests the rolling of leukocytes and induces a tight binding between leukocytes and endothelial cells, allowing them to squeeze between the endothelial cells and extravasate into the affected tissue.

IL-1 and TNF also induce the secretion of chemokines by endothelial cells, which further recruit leukocytes to the inflamed site and amplify the inflammatory response (Isshiki et al, 1990). Chemokines produced by endothelial cells bind to proteoglycan

molecules, both in the extracellular matrix and on endothelial cell surfaces, exhibiting the chemokines on a solid substrate along which leukocytes can migrate. Thus, the role of chemokines in cell recruitment is twofold: to bind to their receptors on leukocytes converting the initial rolling interaction of the leukocytes with endothelial cells into stable binding, and to direct leukocyte migration along a gradient of the chemokine that increases in concentration towards the inflamed site.

Once the leukocytes cross the endothelium and the basement membrane to enter the tissues, their migration to the site of the injury or infection is directed by the gradient of matrix-associated chemokines. The ability of some chemokines, such as IL-8 and MCAF to activate the function of their target cells is also very important. They act on bone marrow endothelium to release neutrophils into the blood that will subsequently migrate, at an enhanced rate, to the inflammation sites.

After entering the inflamed tissue, leukocytes are again activated by cytokines, predominantly IL-1 and TNF, and to a lesser extent, by IL-6. As a result, they start to secrete a whole array of cytokines and inflammatory products, which subsequently amplify the response. This also applies to endothelial cells. Thus, due to the cytokine cascade that is initiated and propagated by IL-1 and TNF, many cell types may be involved in the inflammatory response.

Fever induction:

IL-1, TNF and IL-6 are endogenous pyrogens, raising the body temperature that is believed to help eliminate infections. Fever is induced by the effects of these cytokines

on the thermoregulatory center in the hypothalamus. The cytokine-induced prostaglandin E2 (PGE2) is the second messenger of fever induction. The effects of IL-1, TNF and IL-6 on muscle and fat cells also contribute to fever induction by altering energy mobilization and increasing body temperature. Most pathogens grow better at lower temperatures, and immune responses, such as the processing of antigen are more intense at higher temperatures. At later stages, the effects of IL-1, TNF and IL-6 on the activation of T and B cells, which together with enhanced processing of antigen, increase the adaptive immune response.

The acute phase response:

The liver is the main target of inflammation and the source of essential metabolites that the body needs to overcome stress. It also supplies the necessary components for immediate defense at the site of tissue damage, as well as confining tissue destruction, eliminating harmful agents, and aiding tissue repair. The liver response is also characterized by significant changes in the transport of ions and metabolites, the activities of most metabolic pathways, and the coordinate stimulation of the acute-plasma proteins (APP s). This involves a shift in the proteins secreted by the liver into the blood plasma and is the result of the action of IL-1, TNF and mainly IL-6 on hepatocytes. In the acute-phase response some plasma proteins decrease, while the levels of others markedly increase. The precise function of the acute-phase response is unknown. The increase in opsonizing proteins and anti-proteinases is believed to aid natural immunity and protect against tissue injury, respectively. Direct opsonins enhance the phagocytosis of microorganisms or tissue breakdown products. The C-reactive protein (CRP) binds to the

phophorylcholine portion of certain bacterial and fungal cell wall lipopolysaccharides. When CRP binds to bacteria, it opsonizes them, but also activates the classical complement cascade by binding to C1q. The second APP of interest is mannose-binding protein (MBP). MBP is a calcium-dependent sugar binding protein or lectin, a member of collectins family. MBP binds to mannose residues on many bacteria, acting as an opsonin. Its structure resembles that of C1q of the complement system, and similar to C1q may activate a proteolytic enzyme complex that cleaves C4 and C2 to initiate complement activation. CRP and MBP do not bind to mammalian cell membranes, as phosphorylcholine in membrane phospholipids is in a form that does not react with CRP, whereas mannose residues on mammalian cells are covered with other sugars and cannot bind to MBP. CRP and MBP have the functional properties of antibodies such as enhancing phagocytosis, as they can bind to a broad range of microorganisms, providing defense mechanisms within a day or two after the initiation of injury or infection before specific immunity develops.

Protease inhibitors (α1-antitrypsin, α1-antichemotrypsin, α1-antiplasmin, and plasminogen activator inhibitor-I) neutralize lysosomal hydrolases released from macrophages and neutrophils and limit tissue damage.

Pro-inflammatory cytokines are the major inducers of APP synthesis, mainly through the activation of gene transcription in the hepatocytes.

In conclusion, the result of early activation of macrophages, with some contributions from platelet aggregation and the secretion of alarm cytokines (IL-1 and TNF), are the local manifestations of the inflammatory response.

RESOLUTION OF INFLAMMATORY REACTIONS:

The net amount of pro-inflammatory cytokines, especially the "alarm cytokines" (IL-1 and TNF) and the duration of their secretion determine the nature of the inflammatory response. Low amounts of cytokines usually result in local inflammation, while high amounts of IL-1 and TNF may result in septic shock and death. The generation of local low-moderate amounts of IL-1 and TNF for extended periods also contributes to the pathogenicity of autoimmune processes, as in rheumatoid arthritis and other organ-specific autoimmune diseases.

Pro-inflammatory cytokines, especially IL-1 and TNF, also participate in tissue repair and wound healing. These cytokines stimulate cells (phagocytes, fibroblasts, chondrocytes and other stromal cells) to generate and secrete degradative enzymes such as metalloproteinases necessary to mediate tissue remodeling for subsequent phagocytosis by tissue macrophages, also stimulated by IL-1 and TNF. In addition, IL-1 and TNF stimulate fibroblast proliferation and deposition of extracellular matrix constituents (ECM), contributing to scar formation.

Acute inflammatory responses are usually limited by the decay in the initiation events, which usually also results in the cessation of synthesis of pro-inflammatory cytokines. In addition, there are inflammation-mediated mechanisms, which actively suppress pro-inflammatory cytokine synthesis. IL-1 and IL-6 act on the adrenal-pituitary axis to generate adrenocorticotropic hormone (Fantuzzi, 1994) that induces the production of cortisol which inhibits cytokine gene expression. The IL-1 receptor antagonist (IL-1Ra) (22kD) is of special interest as a physiological inhibitor that binds to

IL-1 receptors (IL-1R) without inducing agonistic effects. It is produced by several cells usually in conjunction with IL-1, functioning as a natural inhibitor. It has recently been shown that IL-1Ra fails to bind to the IL-1 receptor accessory protein (IL-1RAcP), a signal transducing chain that binds to complexes of IL-1 bound to its receptor, and thus fails to transmit the activation signal. Soluble cytokine receptors, which limit the amount of IL-1 and TNF in the various microenvironments in the body, have been characterized. Soluble receptors may originate from proteolytic cleavage of cell surface receptors or from alternative splicing of receptor encoding mRNAs.IL-1, one of the two types of receptors for IL-1, acts as a decoy target binding to IL-1 but not leading to signal transduction. Disturbances in the ratio between the cytokine and its natural inhibitors have been characterized in various pathological cases, such as IL-1 and TNF in septic shock and chronic inflammatory diseases (i.e. RA) (Dinarello, 1996). In these pathological conditions, excess of the cytokine in comparison to its natural inhibitors is observed. In addition, anti-inflammatory cytokines, such as IL-4 and IL-10, IL-13 and TGFβ inhibit the production of pro-inflammatory cytokines. These cytokines are mainly generated by TH 2 cells, macrophages and other stromal cells.

Changes in levels of acute phase proteins are associated with increased plasma levels of some indicators of micronutrient status, such as ferritin, and decrease of others, such as retinol. Alterations in the plasma levels of acute phase proteins can occur from hemodilution, sequestration and increased or decreased rates of synthesis and breakdown. How much these relate to functional deficiency is not known. Assays that are less perturbed by inflammation, such as the transferrin receptor assay, and adjustment of plasma micronutrient levels according to different cutoff levels for acute phase proteins

are helpful but they do not enable precise assessment of micronutrient status among individuals who are infected. Improving assessment of micronutrient status is important if micronutrient interventions are to be targeted to those with the greatest need.

Assessing micronutrient status in human samples is difficult. Assays may be made for micronutrients in body fluids, such as serum, plasma or breast milk; tissues such as red blood cells and their binding or transport proteins; or measurement of micronutrient-dependent enzymatic activities. Many factors affect micronutrient levels, and plasma levels of several important micronutrients fluctuate considerably after meals. They change during the hemodilution at certain stages of pregnancy and are influenced by exercise. The most marked changes occur during the inflammatory processes of infection (Beisel et al, 1971).

The effect of inflammation on micronutrient status has been recognized for many decades. The classic publication Interactions of Nutrition and Infection by Scrimshaw, Taylor and Gordon in 1968, reviewed what was known about the effect of inflammation on vitamin A, thiamin, riboflavin, ascorbic acid, vitamins D and K, iron, zinc and copper. Two lines of evidence were explored in that review. The first concerned the association between severe clinical infections and low plasma levels of micronutrients. Despite the close interaction between micronutrient malnutrition and inflammation, in which it is often, rather difficult to know which the prime driver is, the authors identified reports of sequential measurements in infected individuals that showed a key role for inflammation as a primary cause of changes in levels in biological fluids. The second line of evidence was from animal and human volunteer experiments in which infections or inflammation

were introduced under controlled conditions and micronutrient levels followed at different stages of the disease process. Subsequently, the work of Beisel et al (1995) identified the time course of changes in micronutrient levels during detailed experimental infections.

Although there were striking changes in micronutrient levels during the clinically apparent illness and during the periods of peak pyrexia, important changes also occurred during the incubation and convalescent periods when pyrexia and clinical illness were not present. This indicated that sub clinical infections also played a key role in influencing micronutrient status (Hurt et al, 1994).

Most of these studies relied on measurements of micronutrients in plasma or urine. They gave important information on the overall relationship between inflammation and micronutrient malnutrition.

The characteristics of the biochemical and immunological response to infection are now reasonably well characterized. The term "acute phase response" is used to describe a short-term metabolic change evidenced by increased plasma concentrations of certain proteins—positive acute phase proteins (APP s)—such as C-reactive protein (CRP), haptoglobin, fibrinogen and α–1 antitrypsin and decreased concentrations of certain proteins—negative APP s—such as albumin, retinol binding protein (RBP), transthyretin (TTR), and high-density lipoprotein-apolipoprotein A1 (DHL-apo A), which tend to fall during infection (Fleck, 1989). This is associated with a wide range of changes in circulating levels of cytokines and immunoglobulins. The metabolic changes

are often supplemented by physiological changes such as altered pulse rate, temperature and blood pressure.

In recent years the development of reactive oxygen species has been described during the acute inflammatory phase of many illnesses and experimental studies. This development leads to oxidative stress in which there is increased use of antioxidants such as vitamins C and E, selenium and carotenoids, with a reduction in plasma levels. There seems to be an increased consumption of antioxidants leading to lower plasma levels but whether changes in rates of turnover of these antioxidants compensates for their lower levels in plasma is not yet clear (Allard et al., 1994).

Iron:

The lability of serum iron during infection is well known. In view of the ubiquitous requirement for iron by microbes infecting humans, it is just as well that the inflammatory response reduces levels of free circulating iron and increases levels of circulating of binding proteins. Many studies show the marked elevation of ferritin during the acute and chronic phases of inflammation (Witte, 1991). When ferritin levels are low (e.g., <10 μg/L), there can be little doubt that iron status is deficient. However when inflammation is present, ferritin levels may often be >20 μg/L even in the presence of marked iron deficiency, as assessed by red blood cell indices and plasma ferritin levels when the infection is gone.

More recently the transferrin receptor (TfR) assay has been used because it was hoped that TfR would be more stable than ferritin. Several studies showed an effect of

infection such as malaria on plasma TfR levels; although changes occur during acute infection in non immune subjects, these are considerably less than changes in ferritin.

Zinc:

Zinc status is often assessed by measurements of zinc in plasma, white blood cells or hair. However many dietary and physiological factors such as exercise, eating, pregnancy and rapid growth in childhood may all alter plasma zinc levels. Whether this really represents zinc deficiency is arguable, and Golden (1982) has discussed the criteria for assessing zinc status. Attempts at measuring metallothionein, an alternative indicator of zinc deficiency, have not resulted in robust indicators of assessment. Overall, mean plasma zinc levels in a population can be used to indicate deficiency. Plasma zinc and metallothionein are both reduced during acute phase response In addition there are considerable urinary losses of zinc in systemic infection, particularly those with a pronounced metabolic stress leading to breakdown of muscle. The reduction of circulating zinc reduces zinc availability for microbial metabolism during infection (Isaksen, 2001), providing an advantage similar to that achieved by reducing iron levels. The recent recognition that calprotectin is released (from damaged neutrophils) during inflammation provides another mechanism for reducing plasma zinc levels during inflammation.

The amount by which plasma zinc falls in spontaneous infection was examined in Peruvian children in whom infection was diagnosed according to clinical signs or elevated CRP (Brown, 1998). A plasma zinc difference of around 0.5 m mol/L was noted between infected and non infected children. This contrasted with more striking

differences in mean ferritin levels (10.0 μg/L in uninfected vs. 3.9 μg/L in infected children). An indirect method of assessing zinc status using alkaline phosphatase has been recognized for years. This changes when zinc supplements are given to children but its functional importance is difficult to assess because of the changes in plasma alkaline phosphatase during rapid growth.

Magnesium:

Higher serum magnesium levels are seen in APR patients. It may be attributed to sub clinical renal ischemia and possibly to increased serum glucose level (da Cunha et al, 1999). The development of reduced levels of ionized magnesium while in intensive care was associated with higher mortality and more severe organ dysfunction. Sepsis was an independent risk factor for ionized hypomagnesaemia. Prolonged disease and diuretic administration may also be contributory. It may be that low levels contribute to critical illness, or that just the converse is true. The outcomes of supplementing magnesium in these situations need to be explored (Justin kirk-bayley, 2003). Higher level of magnesium is known to protect against soft tissue calcification which may be the basis for apparent protection that dietary magnesium exerts against myocardial infarction deaths (Sherman bloom, 1989).

How much does inflammation change micronutrient status?

This key question has several components. The first is whether it is possible to measure micronutrient status in an individual even without the presence of inflammation. The studies reviewed here indicate that for certain micronutrients such as folate, assays exist that are robust indicators of nutritional status. For others such as iron, the position is

less satisfactory. The second component concerns the proportion by which micronutrient levels change as a result of the inflammatory process.

There is no linear relationship between change in inflammatory proteins level and change in micronutrient status. Around 10 years ago Brown et al (1993) concluded that the effect of concurrent infections may differ by nutrient, nutritional status of the population and prevalence and severity of the infection.

According to international criteria there are levels of ferritin and retinol at which iron deficiency and vitamin A deficiency, respectively, are recognized. There are clear guidelines for public health nutrition interventions. However, using the available indicators in populations burdened with a high prevalence of infection may lead to an underestimate of some micronutrient deficiencies like iron and an overestimate of others like zinc.

AIM AND OBJECTIVE

AIM AND OBJECTIVE

To estimate the levels of trace elements viz., zinc, magnesium and iron

in viper envenomation induced acute inflammatory status

and

compare with the control population.

MATERIALS AND METHODS

MATERIALS AND METHODS

Setting: Department of medicine, Rajaji hospital and Madurai medical college, Madurai.

Design of study: analytical study.

Period of study: October 2003 to September 2004.

Ethical committee approval: The present project was approved by the ethical committee.

Criteria for selection of subjects:

Rigid criteria were adopted for inclusion and exclusion of cases and controls for the present study. The details are furnished below.

Inclusion criteria:

1. Patients who were brought to the hospital with a history of viper bite and developed features of envenomation.
2. Patients who developed complications of envenomation which did not have a direct effect on trace element levels in the serum.

Exclusion criteria:

1. Patients with history of snake bite but had not identified the snake.
2. Patients with history of snake bite without features of envenomation.
3. Bites other than viper.
4. Previous envenomation and administration of ASV.
5. Patients having underlying hematological disorders.
6. Patients having previous kidney disease.
7. Patients having any other inflammatory pathology.
8. Patients having evidence of immune and nutritional deficiency.
9. Patients on multivitamin supplementation.

10. Patients who had received any other (native) treatment for envenomation.
11. Patients who were not willing to participate in the study.
12. Pregnant and lactating women.
13. Patients who were treated for any other infections during the past 15 days.
14. Patients who were unconscious, in shock, developed acute renal failure.
15. Patients who were administered diuretics.
16. Children below 12 years.

Consent:

Informed consent was obtained from all those who participated in the study.

Materials:

Thus a total of 20 cases that satisfied the inclusion and exclusion criteria stated above were taken up for the study. A total of 20 age and sex matched subjects who did not have any underlying nutritional, inflammatory, renal or hematological pathology as per the exclusion criteria, were included as control.

Definitions:

The definitions adopted during the study period with reference to selected entities are furnished below.

1) Envenomation:

Patient was considered envenomated if he/she had evidence of cellulitis and regional adenitis and with one or more hematological manifestations of snake bite.

2) Non specific complaints:

Patients who had symptoms due to fear and anxiety like palpitation, headache, giddiness.

3) Native procedures:

It included any of the following invasive and non-invasive procedures. Invasive procedures might be biting and applying suction, incising or cauterisation over snake bite site. Noninvasive procedures included topical application of ice, $KMnO_4$, calcium hydroxide or green leaves extract or oral administration of green leaves extract.

4) Tourniquet application:

Tourniquet application was taken into account if it was tight enough to occlude arterial or venous blood flow using nylon rope, which forms a tight constriction band.

6) Fang marks:

Presence of two prominent bite marks was taken into account as the impression of the fangs. Multiple bite marks that showed the impression of the teeth of the snake were not considered as fang marks.

7) Compartment syndrome:

This included the presence of neurovascular deficit, in the affected limb due to the spread of inflammation beyond the subcutaneous plane into the muscular compartment.

8) Complications during hospital stay:

It constituted mainly the infective complications as urinary tract infection, respiratory tract infection and secondary infection of any skin lesions if present. It also included complications of invasive procedures undertaken while treating snake envenomation.

Methods:

Selected sociodemographic, clinical and laboratory data were collected from the patients and controls and recorded in a pro forma.

Socio demographic data comprised of:

- age
- sex
- locality
- occupation
- place of bite
- time between bite and admission
- native procedures
- application of tourniquet

Clinical data comprised of:

- cellulitis
- urine output
- mucocutaneous bleed
- systemic examination

Laboratory data included:

- Clotting Time
- Urine - albumin, deposits, RBC s, casts.
- Sugar
- Urea
- Creatinine
- Electrolytes
- ECG
- Blood grouping and typing

- Serum zinc
- Serum magnesium
- Serum iron

Clotting time was measured using a clean and dry ampoule. 1 ml of blood was taken by vene puncture immediately after admission and poured into the ampoule. The ampoule was slightly tilted at the end of 20 minutes. If the blood had not clotted till then, it was taken as non clotting one. If it was < 20 minutes, the procedure was repeated after 6 hours. It was also repeated 6 hours after the administration of ASV.

Urine analysis was completed by adopting standard bedside procedure.

Blood sugar and urea, and serum creatinine and electrolytes were estimated using ERBA XL 300 automated analyzer.

Blood grouping and typing was done in required cases using standard slide method.

ECG: 12 lead ECG was taken in all the patients.

For estimating the serum levels of zinc, magnesium and iron, blood was taken by vene puncture within 24 hours of snake bite. Vene puncture site was cleaned properly and blood was collected in clean disposable tubes available in the market. The sample was immediately transported to a quality controlled laboratory where the sample was analyzed using atomic absorption spectrometry. The instrument was started up. After start up, a pipette appeared from the instrument. Blood sample was fed to the instrument by the principal worker. The pipette drew the necessary amount of serum and withdrew on its own. Atomic absorption spectrometry used an atomizer to produce atoms from the sample. It also had light sources from hollow cathode lamps each for a different element

which were housed in a rotating turret so that the correct lamp can be quickly selected. There was also an optical system and detector where a monochromator was used to select the specific wavelength of light which was absorbed by the atom and to exclude other wavelength which allowed the determination of selected element in the presence of others. A calibration curve was used to determine the concentration of the element in the sample. The instrument was calibrated using several solutions of known concentrations. It was continuously rescaled as more concentrated solutions were used; the more concentrated solutions absorbed more radiation up to a certain absorbance. The calibration curve showed the concentration against the amount of radiation absorbed. The sample solution was fed into the instrument and the concentration of element was then displayed on the calibration curve. The instrument was standardized for quality control repeatedly.

Conflict of interest:

There was no conflict of interest.

Financial support:

Nil.

Limitations:

1) The levels of the trace elements after complete recovery had not been measured due to ethical reasons.
2) Whether the changes in the serum levels of the elements were absolute or due to sequestration had not been elucidated.
3) The probable mediators for the alteration in trace elements could not be identified due to technical constraints.

4) Heterogeneity of the patients based on their phenotype and genotype could alter the inflammatory response.
5) Pre envenomation levels, which might depend on their food habits and socio environ, could not be assessed in view of the unnatural illness.

Statistical analysis:

Data were entered in Microsoft Excel spread sheet and analyzed utilizing the software - epidemiological information package 2002 (Epi Info 2002) - developed by centre for disease control and prevention, Alaska for World Health Organization. Range, mean, standard deviation and 'p' values were calculated using this package. Paired't' test was done to find out the significance of relationships between the groups. Significance was considered if the 'p' value was below 0.05.

RESULTS

RESULTS

The total number of subjects included in the study was 40. Among the 40 subjects, 20 were cases and 20 were controls and their profile is furnished below.

Age:

The age of the cases ranged from 15 to 75 years and that of controls ranged from 20 to 56. The mean and standard deviation for the cases were 38 ± 16.27 years and those for the controls were 34.5 ± 9.82 years. Among the cases the mean age of males was 43 ± 19.44 years and that of females was 33 ± 11.22 years. A comparative analysis did not show statistically significant difference ('p'=0.3408).

Sex composition:

Among 20 cases studied, there were 10 males and 10 females. Among controls, there were 10 males and 10 females. The differences in the sex composition among cases, and between cases and controls were not significant.

Relationship between serum trace elements among total cases and controls:

The serum zinc value in the cases was 54.23 ± 29.61 µg/dl and that of controls was 117.13 ± 42.28 µg/dl. A comparative analysis of these values showed a statistically significant difference ('p'=0.0001).

The mean value of serum magnesium in the cases was 2.28 ± 0.5 mg/dl and that of controls was 2.12 ± 0.37 mg/dl. The value of serum iron in the cases was 90.2 ± 50.03

µg/dl and that of controls was 117.27 ± 42.36 µg/dl. A comparative analysis of the values of serum magnesium ('p'=0.4277) and iron ('p'=0.2093) among cases and controls did not show any statistically significant difference. The details are depicted in the table-1 given below.

Table-1

Serum level of zinc, magnesium and iron in cases and controls

	Case Mean(SD)	Control Mean(SD)	t	p
Zinc (µg/dl)	54.23(29.61)	117.13(42.28)	3.8138	0.0001
Magnesium (mg/dl)	2.28(0.5)	2.12(0.37)	0.8016	0.4277
Iron (µg/dl)	90.2(50.03)	117.27(42.36)	1.2771	0.2093

Relationship between serum trace elements among male cases and controls

The serum zinc level in male cases was 61.59 ± 26.89µg/dl and that in male controls was 143.55 ± 23.97µg/dl. A comparative analysis of these values showed a statistically significant difference ('p'=0.0001).

The serum level of magnesium among male cases was 2.38 ± 0.65 mg/dl and that among male controls was 2.09 ± 0.38 mg/dl. The iron level among male cases was 101.5 ± 50.53 µg/dl and among control males was 124.85 ± 41.79µg/dl. A comparative analysis between serum magnesium ('p'=0.4094) and iron ('p'=0.4578) among male cases and

controls did not show any statistically significant difference. The details are depicted in table-2 given below.

Table-2

Serum level of zinc, magnesium and iron in male cases and controls

	Case Mean(SD)	Control Mean(SD)	T	p
Zinc (µg/dl)	61.59(26.89)	143.55(23.97)	4.8344	0.0001
Magnesium (mg/dl)	2.38(0.65)	2.09(0.38)	0.8447	0.4094
Iron (µg/dl)	101.5(50.53)	124.85(41.79)	0.7588	0.4578

Relationship between serum trace elements among female cases and controls

The serum zinc level in female cases was 47.45 ± 31.88 µg/dl and that in female controls was 90.7 ± 40.58 µg/dl. A comparative analysis of these values showed a statistically significant difference ('p'=0.0402).

The serum level of magnesium among female cases was 2.2 ± 0.29 mg/dl and that among female controls was 2.16 ± 0.37 mg/dl. The iron level among female cases was 78.9 ± 49.47 µg/dl and among control females was 109.69 ± 43.75 µg/dl. A comparative analysis between serum magnesium ('p'=0.8578) and iron ('p'=0.3349) among female cases and controls did not show any statistically significant difference. The details are depicted in table-3 given below.

Table-3

Serum level of zinc, magnesium and iron in female cases and controls

	Case Mean(SD)	Control Mean(SD)	t	p
Zinc (µg/dl)	47.45(31.88)	90.7(40.58)	2.7906	0.0402
Magnesium (mg/dl)	2.2(0.29)	2.16(0.37)	0.1818	0.8578
Iron (µg/dl)	78.9(49.47)	109.69(43.75)	0.9909	0.3349

Relationship between serum trace elements among male and female cases:

Among the cases the mean serum values of zinc, magnesium and iron did not show statistically significant difference when compared between males and females ('p' > 0.05). The details are depicted in table-4 below.

Table-4

Serum level of zinc, magnesium and iron in male and female cases

	Male Mean(SD)	Female Mean(SD)	t	p
Zinc (µg/dl)	61.59(26.89)	47.45(31.88)	0.7192	0.4812
Magnesium (mg/dl)	2.38(0.65)	2.2(0.29)	0.5745	0.5728
Iron (µg/dl)	101.5(50.53)	79.8(49.47)	0.6735	0.5092

Relationship between serum trace elements among male and female controls:

Among the controls the mean serum values of zinc, magnesium and iron did not show statistically significant difference when compared between males and females ('p' > 0.05). The details are depicted in table-5 below.

Table-5

Serum level of zinc, magnesium and iron in male and female controls

	Male Mean(SD)	Female Mean(SD)	t	p
Zinc (μg/dl)	143.55(23.97)	90.7(40.58)	0.6804	0.4613
Magnesium (mg/dl)	2.07(0.38)	2.16(0.37)	0.5752	0.5771
Iron (μg/dl)	124.85(41.79)	109.69(43.75)	0.6145	0.5386

Relationship between time of bite and admission, and trace element levels:

The serum levels of zinc, magnesium and iron did not show statistically significant difference due to delay in reaching the hospital for management ('p' > 0.05). Table-6 shows this detail. The mean time period between the time of bite and admission to the hospital was 6.13 $\pm$ 10.53 hours and the time of starting ASV to the patients was 9.53 $\pm$ 10.5 hours. The reduction in serum zinc level among the patients with envenomation was significantly lower than healthy control irrespective of the interval between bite and sample collection.

Table-6

Serum trace element level and time lapse for admission to hospital:

	< 2 hrs (n=11) Mean(SD)	2-6 hrs (n=6) Mean(SD)	> 6 hrs (n=3) Mean(SD)	t	p
Zinc (µg/dl)	52.84(31.25)	65.98(26.68)	46.83(35.26)	0.5195	0.6194
Magnesium (mg/dl)	2.85(0.45)	2.38(0.74)	2.23(0.29)	0.2799	0.7877
Iron (µg/dl)	83.47(53.7)	114.62(49.47)	81.8(30.97)	0.7455	0.4802

Relationship between tourniquet application and trace elements:

The relationship between application of tourniquet and the alterations in the levels of zinc, magnesium and iron were studied. There was no statistically significant difference which was shown by 'p' > 0.05. The following table shows this detail.

Table-7

Serum trace element level and application of tourniquet:

	Tourniquet Applied(n=12) Mean(SD)	Tourniquet Not applied(n=8) Mean(SD)	t	p
Zinc(µg/dl)	49.3(27.07)	62.(33.33)	0.6296	0.5369
Magnesium (mg/dl)	2.25(0.62)	2.3(0.26)	0.1753	0.8628
Iron (µg/dl)	88.31(60.88)	93.03(30.89)	0.1532	0.8769

Relationship between time of onset of cellulitis and serum trace element levels:

The time of onset of cellulitis and the serum levels of zinc, magnesium and iron was studied. There was no statistically significant difference as 'p' is > 0.05. Table-8 shows this detail. The average time of onset of cellulitis was 72.75 ± 133.94 minutes.

Table-8

Serum levels of trace elements and time of onset of cellulitis

	< 60 min(n=14) Mean(SD)	>=60 min(n=6) Mean(SD)	t	p
Zinc (µg/dl)	54.22(31)	55.23(28.83)	0.0470	0.9630
Magnesium (mg/dl)	2.21(0.57)	2.44(0.25)	0.8522	0.4053
Iron (µg/dl)	86.68(53.99)	98.4(42.61)	0.3444	0.7345

Relationship between extent of cellulites and serum trace elements levels:

The serum level of zinc in patients who had developed cellulitis over the entire limb in which bite had acquired was 25.82 ± 5.57 µg/dl, whereas that in patients who had developed cellulitis was 66.84 ± 26.97 µg/dl. Comparing these two values showed statistically significant difference ('p'= 0.0001), thus indicating the fall in serum zinc level was significantly associated with the nature/manifestation of inflammatory response.

Similarly the serum iron values also showed statistically significant difference of 'p' = 0.0165, but serum magnesium did not show statistically significant difference ('p' > 0.05). These details are shown in table-9.

Table-9

Serum zinc, magnesium and iron, and extant of cellulitis:

	Part of limb(n=15) Mean(SD)	Whole limb(n=5) Mean(SD)	t	p
Zinc (µg/dl)	66.84(26.97)	25.82(5.57)	4.1049	0.0001
Magnesium (mg/dl)	2.17(0.46)	2.64(0.54)	1.1961	0.2472
Iron (µg/dl)	109.36(46.71)	47.88(21.57)	0.2472	0.0165

Relationship between the presence of mucocutaneous bleed and trace elements:

The relationship between the presence of mucocutaneous bleed and serum levels of trace elements was studied. Observations did not show any statistically significant difference in their levels when compared between the patients with and without mucocutaneous bleed as the 'p' value was > 0.05. These details are shown in table-10 below.

Table-10

Mucocutaneous bleed and trace element level

	Mucocutaneous bleed present(n=16)Mean(SD)	Mucocutaneous bleed absent (n=4) Mean(SD)	t	p
Zinc (µg/dl)	48.33(26.33)	79.27(24.94)	1.1254	0.2872
Magnesium (mg/dl)	2.27(0.53)	2.29(0.18)	0.1405	0.8591
Iron (µg/dl)	80.22(45.5)	130.07(40.3)	1.0627	0.3516

Relationship between bleeding from fang marks and trace elements:

Comparing the levels of trace elements among patients with and without bleeding from the fang marks did not show any statistically significant difference as the 'p' value was > 0.05. Table-11 shows these details.

Table-11

Fang mark bleed and trace element level

	Fang mark bleed present(n=16) Mean(SD)	Fang mark bleed absent(n=4) Mean(SD)	t	p
Zinc (µg/dl)	44.91(28.14)	48.97(24.95)	0.2376	0.8149
Magnesium (mg/dl)	2.27(0.55)	2.33(0.32)	0.1295	0.9253
Iron (µg/dl)	90.45(45.27)	102.32(42.35)	0.3742	0.7581

ASV vials per patient:

The total number of vials of ASV given per patient on an average was 14.4 ± 7.17.

Total days of inpatient stay:

On an average each patient stayed for a period of 3.95 ± 0.94 days in the hospital.

There were no deaths, and none of them underwent fasciotomy or hemo dialysis. Also none required mechanical ventilation.

DISCUSSION

DISCUSSION

Snake bite is an important health hazard in developing countries like India. As the victims of snake bite are mostly healthy and belong to the economically productive age group, the study about inflammation and its effects in the human body is essential in order to introduce specific or supportive therapy or modifying the current therapeutic programme.

Among these 20 cases studied, the mean age of the patients who suffered from snake bite was 38 $\pm$16.25 years, with a range of 15 to 75 years. This is in par with the observations of Sawai and Homa (1974), who observed a range of 11 to 50 years.

Regarding the sex distribution previous studies had shown a 3:1 ratio among males and females (Sawai and Homa, 1974), but in the present study male and female ratio was 1:1 because of the criteria adopted for inclusion and exclusion.

Snake bite and envenomation is a major health hazard mainly in the rural areas. In the present study 75% of the cases were from rural areas. Similarly 95% of our cases were agricultural workers. All the bites had been acquired in outdoor environment, during the working hours from morning 6 am to evening 6 pm, except only one patient who acquired the bite during the mid night.

80% of the bites were over the lower limbs especially on the foot. This shows that wearing of shoes might have protected them. However people who work in the fields were not accepting to wear a shoe, as they feel that the agricultural lands are equivalent to

holy places and wearing a shoe while working is a sin. All other bites were over the hands, where wearing a glove could have protected them.

Envenomation does not depend on the age and size of the snake (Kochva, 1978). Even though the snake might have swallowed a prey just before bite, there is a possibility of envenomation. In the present study also a person with features of envenomation was bitten by a small snake which had swallowed two rats.

30% of the patients had nonspecific complaints mainly due to fear and anxiety. The commonly encountered symptoms were headache, vomiting, giddiness and sweating. The primary task was to differentiate this from neurotoxicity, but none developed neurotoxicity in the present study.

Skin lesions developed in only two of our patients in the form of cutaneous bleb going for ulceration and the other developed hematoma with skin discolouration. There was no significant correlation between skin lesion and the intensity of envenomation. None developed compartment syndrome.

All the patients had prolonged clotting time (> 20 min). All had normal ECG. Only one patient was transfused with fresh blood, as she developed massive UGI bleed. None of them underwent any surgical procedure or dialysis or mechanical ventilation. On an average 14.4 vials of ASV was given per patient. Among them seven patients developed reactions to ASV. The common symptoms were itching and rash. Two of them had fever with chills and rigors, and the other one had vomiting. One of the patients had hypotension and shock, and was resuscitated with adrenaline. The serum samples

were taken before the administration of ASV and so the allergic reactions might not have any effect on the trace elements estimated here.

The duration of stay in the hospital on an average was four days. As the duration was less, there were no significant complications during stay.

Studies show alterations in the serum levels of trace elements during acute inflammation. The mechanisms by which these alterations occur have not been elucidated as also the effects of these alterations. Various hypotheses have been put forth for these alterations like changes in renal handling of these elements by the actions of cytokines which are the key mediators of inflammation (Beisel et al, 1995). The other one is sequestration of these elements from serum into tissues of the body. This supports the view that, tissue estimate of trace elements by biopsy should have been done along with serum levels, to assess their alterations during inflammation. Similarly, sequential measurement is necessary to assess the changes in their levels (Scrimshaw, Taylor and Gordon, 1968).

The effects of these changes may be a protective auto regulatory mechanism to minimize the damage produced by inflammation. For example, reduction in serum zinc and iron levels decreases the vigor of inflammation due to infection where iron serves as an enrichment medium for growth and multiplication of organisms (Gordeuk et al, 2001). Similarly it decreases the formation of free radicals. A zinc deficient state may also possibly decrease the action of zinc dependent metallothionines or metalloenzymes.

The increase in serum magnesium levels also carries similar values as shown by Sherman bloom (1989), that it reduced the inflammation and calcification of myocytes after acute myocardial infarction which is also an acute inflammatory state.

Even though the therapeutic implications of the serum levels of these elements have not been worked out, they at least predict the brunt of inflammation occurring inside the body. So they can be taken as a marker of acute inflammation.

In the present study, an attempt was made to find out the association between the alterations in the serum levels of trace elements in viper envenomation. There were not much works in the evaluation of trace element levels in viper envenomation.

The present study showed statistically significant decrease in the serum levels of zinc during envenomation induced inflammation, which goes parallel with the results of zinc in the setting of infection induced inflammation by Brown (1998). This was true while comparing total cases with controls irrespective of gender. Difference in the level of serum iron and magnesium between cases and controls was not significant where as Brown (1998) observed a significant increase in levels of ferritin among patients suffering from an acute infection. Ferritin is an iron binding protein which is increased in inflammation which secondarily causes a decrease in iron levels.

Development of cellulitis with lymphadenitis is considered an impending sign of systemic envenomation. It may also be due to infection produced by microbial agents injected during snake bite. The values among patients who developed cellulitis within 1 hour and those who developed cellulitis after the first hour of bite, which showed the

rapidity of onset of inflammation, was compared. However there was no significant difference in zinc, iron and magnesium levels with reference to time interval between bite and sample collection. Serum zinc levels of patients who developed massive cellulitis involving the entire limb when compared with those who had only minimal cellulitis, which showed the magnitude of inflammation, showed a significant decrease in the levels of zinc and iron, but the levels of magnesium did not show any significant difference. The association with inflammation indicates that inflammation induced acute phase reactant proteins might have contributed for these alterations.

Twelve patients had tourniquet during admission. Among the patients who had cellulitis involving the entire limb, 4 had applied tourniquet. This shows that tourniquet application has no protective value in the treatment of snake bite. Also no one had compartment syndrome due to application of tourniquet, contrary to the belief.

Like regional adenitis, persistent bleed from the fang marks is an important marker of envenomation and hemotoxicity. Comparing the results of serum levels of trace elements between those who had bleed from the fang marks and those who did not have bleed from the fang marks there was decrease levels in those with bleed but did not show any statistical significance.

80% of the patients had hematological manifestations in the form of mucocutaneous bleed. Of them, the major site of bleed was from the gum which is correlating with the literature (Hutton and Warrell, 1993), and examination of oral cavity thoroughly is a must. Four out of twenty (20%) had hematemesis, and 6(30%) had

hematuria. There was no statistically significant difference between those with mucocutaneous bleed and those who did not.

In summary, the inflammatory response is markedly influenced by the release of cytokines, such as TNFα and IL-1 induced by the venom metalloproteinases (Laing et al, 1997). Subsequently, the cytokines activate the endogenous metalloproteinases in various cells (fibroblasts), which can cleave TNFα and amplify the process of cell destruction and necrosis, which in turn alter serum trace elements. Thus the alteration of serum trace elements acts as a biomarker of inflammatory response secondary to envenomation.

Areas of further work:

1) An experimental model with homogeneity is required under controlled environment to confirm the present observation by administering calculated quantity of venom for all the models at the same time.
2) Analysis should be done at periodic intervals to assess the time taken for alteration or for reversal of trace element levels.
3) Tissue levels and urinary levels of trace elements should be studied.
4) Attempts to find out the basic sequence of reactions that result in alteration in trace elements should be undertaken.
5) By keeping the levels of trace elements in the normal range, the variations in the outcomes should be studied to find out whether the alterations in their levels are beneficial for homeostasis during inflammation.

CONCLUSION

CONCLUSION

1) Serum zinc level was significantly lower in patients with snake envenomation when compared with controls and it was independent of gender.
2) Patients who had massive cellulitis had significant decrease in the levels of serum zinc and iron when compared with patients who had minimal cellulitis.
3) Serum levels of zinc, magnesium and iron were found to be independent of the onset of cellulitis, time period between snake bite and admission, presence of mucocutaneous bleed, fang mark bleed and application of tourniquet.
4) Tourniquet application did not restrain the spread of cellulitis.
5) Serum magnesium and iron did not show any significant difference among the patients and controls in all the parameters taken for analysis except for a massive cellulitis when compared to patients with minimal cellulitis.

SUMMARY

SUMMARY

Viper envenomation produces variable inflammatory response. Hence this study on 'Trace elements profile in viper envenomation' was conducted to assess the levels of trace elements in those who had acute inflammation following envenomation.

After institutional ethical clearance, with an informed consent and with rigid criteria, 20 patients were selected carefully and were evaluated on social, clinical and laboratory aspects. Twenty healthy subjects of their own family were included as control. The data were entered in Micro soft Excel spread sheet and analyzed statistically.

There were 10 males and 10 females in both patient and control group. The mean age of the patient group (38±16.25 years) and control group (34.5 ± 9.82) did not differ significantly. The serum level of zinc was significantly lower in patients when taken as a whole or when compared separately among males and females. Also, no significant difference among gender in the patient and control group with reference to zinc was noted. Significant decrease in zinc level in patients who had massive cellulitis was observed. Serum magnesium and iron levels did not show any significant difference among the patients and controls in all the parameters taken for analysis, except for a decrease in serum iron in patients with massive cellulitis. Though there was a decrease in the levels of serum trace elements in patients when compared to controls, while taking into account the onset of celulitis, time period between bite and admission, presence of mucocutaneous bleed, fang mark bleed and application of tourniquet, this difference was not statistically significant.

From the present study, it is concluded that serum zinc level is significantly low in patients with viper envenomation, and at the same time there was no significant difference in the levels of magnesium and iron in them. This decrease correlates positively with the extent of cellulitis and not with that of onset of cellulitis, time between bite and admission, presence of bleed and application of tourniquet. Serum magnesium and iron levels did not show much correlation with the parameters taken for this study.

Measuring serum zinc during acute envenomation will help as a bio-marker to assess the covert inflammatory response or assist in decision making for therapy or prognosis, or guide to predict the clinical course remains to be studied. Hence, further animal models and clinical studies on these areas are recommended not only to find out this usefulness but also to identify the possible triggering or contributing underlying acute phase reactant protein.

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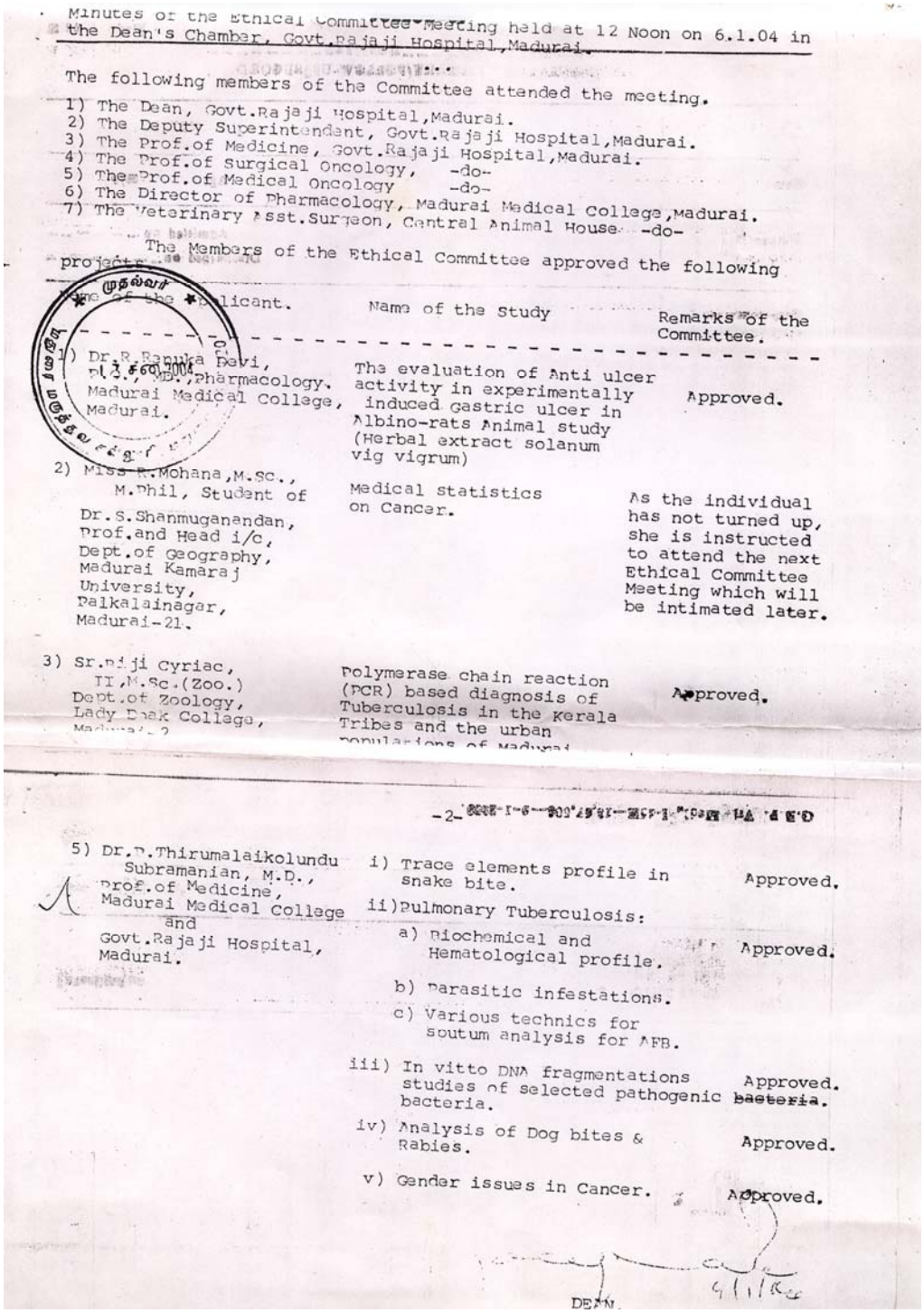
APPENDIX I - APPROVAL FROM ETHICAL COMMITTEE

APPENDIX I

APPROVAL FROM ETHICAL COMMITTEE



Minutes of the Ethical Committee meeting held at 12 Noon on 6.1.04 in the Dean's Chamber, Govt.Rajaji Hospital,Madurai.

The following members of the Committee attended the meeting.

1) The Dean, Govt.Rajaji Hospital,Madurai.
2) The Deputy Superintendent, Govt.Rajaji Hospital,Madurai.
3) The Prof.of Medicine, Govt.Rajaji Hospital,Madurai.
4) The Prof.of Surgical Oncology, -do-
5) The Prof.of Medical Oncology -do-
6) The Director of Pharmacology, Madurai Medical College,Madurai.
7) The Veterinary Asst.Surgeon, Central Animal House -do-

The Members of the Ethical Committee approved the following projects.

Name of the Applicant.	Name of the Study	Remarks of the Committee.
1) Dr.R.Renuka Devi, M.D., Pharmacology. Madurai Medical College, Madurai.	The evaluation of Anti ulcer activity in experimentally induced Gastric ulcer in Albino-rats Animal study (Herbal extract solanum vig vigrum)	Approved.
2) Miss R.Mohana,M.Sc., M.Phil, Student of Dr.S.Shanmuganandan, Prof.and Head i/c, Dept.of Geography, Madurai Kamaraj University, Palkalainagar, Madurai-21.	Medical statistics on Cancer.	As the individual has not turned up, she is instructed to attend the next Ethical Committee Meeting which will be intimated later.
3) Sr.Biji Cyriac, II,M.Sc.(Zoo.) Dept.of Zoology, Lady Doak College, Madurai-2	Polymerase chain reaction (PCR) based diagnosis of Tuberculosis in the Kerala Tribes and the urban populations of Madurai	Approved.

-2-

5) Dr.D.Thirumalaikolundu Subramanian, M.D., Prof.of Medicine, Madurai Medical College and Govt.Rajaji Hospital, Madurai.	i) Trace elements profile in Snake bite.	Approved.
	ii) Pulmonary Tuberculosis: a) Biochemical and Hematological profile.	Approved.
	b) Parasitic infestations.	
	c) Various technics for Soutum analysis for AFB.	
	iii) In vitto DNA fragmentations studies of selected pathogenic bacteria.	Approved.
	iv) Analysis of Dog bites & Rabies.	Approved.
	v) Gender issues in Cancer.	Approved.

DEAN

APPENDIX II – PRO FORMA

APPENDIX II

PRO FORMA

TRACE ELEMENT PROFILE IN VIPER ENVENOMATION

Case No:
Name:
Age:
Sex:
IP No:
Address: (rural/urban)
Occupation: (agricultural/nonagricultural)
Place of bite: (indoor/outdoor)
Time of bite: (working hours/ nonworking hours)
Site of bite: (lower limb/upper limb)
Time between bite and admission:
Time between bite and ASV administration:
DOA:
DOD:
Native procedures: (yes/no)
Tourniquet application: (yes/no)

Local symptoms and signs:

- Pain.
- Cellulitis- onset and extant.
- Skin changes.
- Bleeding from fang marks.
- Features of compartment syndrome.

Systemic symptoms and signs:

- Non specific symptoms.
- Mucocutaneous bleed.
- Neurological symptoms and signs.
- Oliguria.

General examination:

- Anemia.
- Jaundice.
- Cyanosis.
- Facial edema, diaphoresis, goose flesh.
- JVP.

Vitals:

- ❖ Pulse rate.
- ❖ BP.
- ❖ Respiratory rate.
- ❖ Single breath count.
- ❖ Urine output.
- ❖ Weight.
- ❖ Temperature.

Systemic examination:

- CVS
- RS
- ABDOMEN
- CNS

Investigations:

- ◊ Hb, TC, DC.
- ◊ Blood grouping and typing.
- ◊ Urea, creatinine and electrolytes.
- ◊ Clotting time.
- ◊ ECG.
- ◊ Urine albumin, RBC & casts.
- ◊ Serum Zinc, Serum Magnésium, Serum Iron.

Treatment:

1. Injection tetanus toxoid.
2. Antibiotics.
3. ASV.
4. Supportive drugs – chlorpheniramine maleate, hydrocortisone, adrenaline, antiemetics and diuretics.
5. Blood/FFP.
6. Dialysis.
7. Ventilatory support.
8. Wound care/fasciotomy.
9. Treatment of reactions to ASV.

Duration of hospital stay:

Complications during hospital stay:

Health education:

APPENDIX III - MASTER CHART

CONTROLS					
Sl. No.	Age	Sex	Se.zinc (µg/dl)	Se.magnesium (mg/dl)	Se.iron (µg/dl)
1	30	1	152.5	1.97	132
2	28	1	167	2.03	101.3
3	36	2	39	1.91	115.9
4	42	2	69	1.88	100.9
5	27	1	158	1.65	155.7
6	40	2	152	1.86	150.2
7	42	2	68	1.68	184.3
8	25	2	63.5	1.9	56.3
9	40	2	72.5	2.72	109.4
10	56	1	180.5	2.33	97.7
11	20	2	94	2.47	75.9
12	41	1	112.5	2.62	65.5
13	37	2	62.5	2.59	65.4
14	45	1	151	1.4	59.6
15	24	2	141.5	2.53	162.7
16	35	1	116	2.5	147
17	22	1	140.5	1.97	157.3
18	43	1	109.5	1.98	184.6
19	27	2	145	2.1	75.9
20	50	1	148	2.4	147.8

APPENDIX III

CASES

Sl. No.	Age (yrs)	Sex	Site of bite	Bite to admission (hrs	Bite to ASV (hrs)	Tourniquet	Cellulitis onset (min)	Cellulitis extant	Fang mark with bleed	Nonspecific symptom	Mucocutaneous bleed	Urine RBC	ASV vials (no.)	Blood or FFP	Reactions to ASV	Days of hospital stay	Se.zinc (µg/dl)	Se.magnesium (mg/dl)	Se.iron (µg/dl)
1	24	1	1	1.5	5	1	25	1	1	2	1	2	20	2	2	4	62.1	1.5	35
2	32	1	1	6.5	12	1	5	2	1	1	1	1	25	2	1	5	32.5	3.4	30
3	30	2	1	2	6.5	2	45	1	1	2	1	2	20	2	2	4	34.3	1.9	75
4	40	2	1	5	10	1	15	1	1	2	1	2	20	2	2	5	92.5	1.7	109
5	30	1	1	5	8	1	25	1	1	1	1	2	15	2	2	4	82.5	1.3	171
6	35	2	1	1.5	4	1	90	1	2	1	1	1	18	2	2	4	27.3	2.1	43
7	55	2	1	2	5	1	40	1	1	2	1	1	20	2	2	4	25.7	2.1	33
8	19	2	1	2	6	2	30	1	2	2	1	1	20	2	2	4	26.5	2.1	66
9	31	2	1	1.5	5	1	30	2	1	1	1	1	20	2	1	7	17.3	2.6	23
10	75	1	2	4	6	2	240	1	1	2	1	2	20	2	2	4	90.5	2.7	98
11	15	2	1	22	25	2	60	2	1	2	1	1	25	1	2	4	28.2	2.4	54
12	75	1	2	46	49	2	600	1	2	2	1	2	5	2	1	3	87.5	2.4	115
13	30	2	2	1	6	2	30	1	1	2	2	2	5	2	2	3	101	2.5	71
14	55	1	2	2	8	2	30	1	1	1	2	2	5	2	2	3	94.3	2.3	135
15	40	2	1	4	5	2	60	1	1	2	2	2	5	2	2	3	37.1	2.4	130
16	45	1	1	3	6	1	60	1	1	2	1	2	10	2	1	4	60.8	2.7	151
17	40	1	1	0.5	3	1	30	2	1	2	1	1	10	2	1	4	26.3	2.9	57
18	31	1	1	1	4	1	10	1	2	2	1	2	10	2	1	4	54.6	2.8	148
19	35	2	1	1	4	1	20	1	1	1	2	2	5	2	2	3	85.2	2	185
20	23	1	1	11	13	1	10	2	1	2	1	1	10	2	1	3	24.8	1.9	76

Sex:	**Site of Bite:**	**Cellulitis extant:**	**Tourniquet:**	**Others:**
1- Male	1- Lower limb	1- Part of the limb	1- Applied	1- Yes
2- Female	2- Upper limb	2- Whole limb	2- Not applied	2- No